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Introduction:

We now know that aging, in large measure, is a consequence of oxidative stress acting on the basic unit of life - the cell. Damage at the molecular level, from the chemical processes of oxidation, causes the machinery of the cell to eventually malfunction. To put it in simplistic terms, our bodies are slowing “rusting” from within.

Managing the aging process, therefore, is a matter of reducing the rate of cumulative oxidative damage to the machinery of the cell. Managing oxidative stress is, in turn, a matter of lifestyle, diet and genetic endowment. To understand how the machinery of the cell operates, how it wears out and breaks down, is to identify the biochemical “trigger” for aging. To understand how the initial damage at the cellular level spreads outward - like ripples in a pond - to affect tissues, organs and, eventually, the organism itself, is to begin to understand the relationship between aging and degenerative disease.[1]

Aging and the degenerative diseases associated with it are attributed basically to the deleterious side attacks of free radicals on cell constituents and on the connective tissues. The free radicals probably arise largely through reactions involving molecular oxygen catalyzed in the cell by the oxidative enzymes and in the connective tissues by traces of metals such as iron, cobalt, and manganese.[2]

Biological Theories of Aging

As many as 300 or more aging theories have been presented in the literature over the past several decades; however, not all have stood up to scrutiny and in-depth scholarly investigation.[3]The major biological theories that attempt to explain the individual differences in aging fit into one of two categories: genetic aging, which presumes that aging is predetermined or programmed, and non genetic aging, which presumes that aging events occur randomly and accumulate with time.[4,5] Four genetic aging theories are programmed aging, somatic mutation, free radical, and neuroendocrine theories. A nongenetic theory is the wear and tear theory. One or multiple theories may explain the aging process and characteristics as a wide range of factors that may affect aging such as genetics, random events, environment, lifestyle, and/or habits.

Genetic Theories

Programmed aging

The premise of the programmed aging theories is that the human body has an inherited internal “genetic clock” that determines the beginning of the aging process. This genetic clock may manifest as a predetermined or limited number of cell divisions,

called the *Hayflick limit* (also known as *replicative senescence* or *cellular senescence*).[6-9] The Hayflick limit does not affect all cells in the body as germ cells (sperm or egg), and cells in some tumors (cancer) seem to divide infinitely.[8,10] The theory of cellular aging explains why many older adults have one or multiple conditions related to decreased or impaired client factors (sensory, neuromusculoskeletal, cardiovascular, respiratory,digestive, metabolic, and reproductive) and why it is rare to find any of these impairments in a young adult.[11] The perception that cellular senescence is not programmed aging of the whole person explains why such conditions are not universal among older adults.[8]

Somatic mutation theory

According to the somatic mutation theory, stochastic (random) chromosomal changes occur as a result of miscoding, translation errors, chemical reactions, irradiation, and replication of errors; these mutations result in changes in the ribonucleic acid (RNA) deoxyribonucleic acid (DNA) code sequences.[8,10],[12] Mutations of the genetic material within a cell can accumulate if the alterations are not repaired when the code is being transcribed (reading process to make the building blocks of proteins).[9] The accumulation of mutations can alter the genetic sequence of a cell in such a way that the "safe guard" to control the proliferation of cellular growth is deactivated, resulting in unrestrained cell division, sometimes leading to tumor genesis and/or cancer.[10,13] Mutations can occur in the expression of the genetic code or the way that the code is read without directly changing the RNA or DNA sequence; the expression of the genetic code is the epigenome, and the mutations are epimutations.[8,14]

Free radical theory

The free radical theory of aging stemmed from the study of unstable atoms in living cells and the damage they caused as they tried to stabilize.[2,15] Free radicals are highly reactive because of the unpaired electron(s) that seek to be paired but, in turn, damage cells, proteins, lipids, and DNA by altering their structures.[16] Free radicals happen naturally in the body whenever metallic ions, enzymes, or cellular materials combine with oxygen and are also introduced into the body through toxins, pollutants, and tobacco smoke. Low-level, free radical damage is theorized to accumulate over time, especially mutations in mitochondrial DNA (mtDNA), resulting in aging characteristics.[16,17] Most organisms have defense mechanisms to limit the effects of free radicals and to repair the damage left behind, but because not all of the repairs can be fixed, the damage accumulates.[12,15] The accumulated mutations of the mtDNA result from a decrease in or loss of function of the natural antioxidant defense layer in the body and cells. However, Harman[17] highlights some studies that show a decrease

in the loss of mtDNA through the consumption of coenzyme Q10 and other antioxidants such as Ginkgo biloba. Ames[18] discusses the importance of nutrient balance, specifically of iron, copper, zinc, vitamin B6,biotin, and pantothenate. Too much of these nutrients tend to increase oxidative stress and mtDNA damage. However, these nutrients are important for mtDNA repair and cellular function, so too little of these nutrients decreases function and the repair of oxidative damage. The direct effect of the free radical theory on aging and dysfunction continues to be questioned and studied, but it is clear that the presence of oxidative damage from free radicals increases through the life span.[15,16]

Neuroendocrine theory

The neuroendocrine theory suggests that the central nervous system is the aging pacemaker of the body.[19,20] Modification of metabolism or reproductive function affects the life span, and the hypothalamus is predicted to be one possible starting point for neuroendocrine-related changes because it influences the regulation of the metabolic and reproductive systems.[20]

Nongenetic Theory

Wear and tear theory

The wear and tear theory proposes that cumulative damage within the body leads to the death of cells, tissues, organs, and, finally, the organism.[21] Wear and tear are natural from living things to inanimate objects, and organisms are able to repair wear and tear.[21] The wear and tear theory is studied with identical twins. Identical twins are nature's natural clones in that they have identical genotypes (genetic information); however, upon closer inspection there are phenotypic (physical) differences.[22] It was found that epigenetic differences were greater between older monozygotic twins than younger pairs, even if external variables were almost identical.[22] Cases in which the time of death varies between twins indicate that environmental factors may be as important as genetic factors in determining life span.[23] Therefore, it can be concluded that internal and external factors play roles in aging and, like other biological aging theories, wear and tear within the body accumulate through the years.[21,22]

Free Radical Theory

The free radical/oxidative stress theory of aging proposes that free radicals resulting from cellular respiration cause cumulative oxidative damage over time, leading to senescent degeneration and death. Functionally, oxidative stress results when the homeostasis between the formation of free radical oxidants and the deactivation of these agents and repair of associated damage through endogenous antioxidant

defenses and repair mechanisms tilts in favor of the free radicals. [23] This state of oxidative stress basically amounts to an electrochemically *positive* or *electron poor* local environment—a generally toxic state. Although, a variety of free radical species exist, the primary biologically relevant radicals are those resulting from oxygen and nitrogen metabolism. [24,25]

Endogenous Oxidant Formation

There are two main sources of oxygen radical generation *in vivo*: mitochondrial sources and non-mitochondrial sources.[26] In mitochondrial respiration, oxidative phosphorylation is the primary pathway for the generation of metabolic energy. Molecular oxygen is a powerful oxidizing agent and, as such, is an excellent terminal acceptor of electrons in the electron transport chain. “In the textbook scheme of mitochondrial respiration, electron transport involves a coordinated four-electron reduction of O2 to H2O.” [25] However, mitochondrial electron transport is imperfect and, thus, a small percentage of electrons engaged in the electron transport chain react with diatomic oxygen, resulting in the superoxide anion. The superoxide anion can, in turn, be converted into hydrogen peroxide and, subsequently, the extremely reactive hydroxyl radical (3-electron reduction).[27]

Non-mitochondrial sources of oxygen free radicals include hydrogen peroxide degradation via the Fenton Reaction, microsomal cytochrome P450 enzymatic metabolism of xenobiotic compounds, phagocytic cell response to inflammation and infection, and perioxisomal beta-oxidation of fatty acids.[25,27] Additionally, many other enzymes are capable of generating oxygen radicals, both in normal physiologic states and under pathological conditions, frequently in tissue-specific fashion.[25,28] For instance,the neuronal degradation of dopamine by monoamine oxidase can yield hydrogen peroxide and has been implicated as a possible cause of Parkinson’s disease.[25,29]

A chemical reaction that usually takes place at ambient temperature between atmospheric oxygen and organic compound is generally considered as autoxidation. Autoxidation phenomena could be completely prevented by total exclusion of oxygen or other oxidizing substances from biological system. This is generally not practical but changes in endogenous factors such as addition of inhibitors may decrease the reaction rate or prolong the induction period. Substances that can suppress autoxidation are referred as inhibitors or antioxidants.[30]

Oxidation commonly involves free radical mechanism. Free radicals are molecules or ions containing unpaired electrons. Reactive oxygen species (ROS) is a term, which encompasses all highly reactive oxygen – containing molecules, including

free radicals. Types of ROS include the hydroxyl radical, hypochlorite radical and various lipid peroxides. All are capable of reacting with membrane lipids, proteins and enzymes and other small molecules, resulting in cellular damage. To protect the cells and organ systems of the body against reactive oxygen species, humans have evolved a highly sophisticated and complex antioxidant protection system. It involves a variety of components, both endogenous and exogenous in origin, that function interactively and synergistically to neutralize free radicals.

Antioxidant protection system:

a) Nutrient: Derived antioxidants like ascorbic acid (Vitamin C), tocopherols and tocotrienols (Vitamin E), carotenoids and other low molecular weight compounds such as glutathione and lipoic acid.

b) Antioxidant enzymes: Superoxide dismutase, glutathione peroxidase and glutathione reductase, which catalyze free radical quenching reactions.

c) Metal binding proteins: Ferritin, lactoferrin, albumin and ceruloplasmin that sequester free iron and copper ions that are capable of catalyzing oxidative reactions.

d) Antioxidant: Phytonutrients present in a wide variety of plant foods[31].

Antioxidants are used to prevent the oxidation of active substances and excipients in the finished products.

Types of antioxidants:

a) True Antioxidants: They act by a chain termination mechanism by reacting with free radicals e.g. butylated hydroxytoluene.

b) Reducing agents: They have a lower redox potential than the drug and get preferentially oxidized e.g. ascorbic acid.

c) Antioxidant synergists: They enhance the effect of antioxidants e.g. EDTA.

- Active principles present in natural products used in anti-aging products include: Vitamin. E, vitamin C, Coenz Q10, lipoic acid, folic acid, flavonoids, Carotenes, Vitamin B complex, Carnitine, Vinpocetine and Melatonin[32]

Literature Review:

Antioxidants have application both in pharmaceuticals to stabilize products with sensitive drugs and in human beings as anti aging agents.

Free radicals and other reactive oxygen species are constantly formed in the human body. Many of them serve useful physiological functions, but they can be toxic when generated in excess and this toxicity is often aggravated by the presence of ions of

such transition metals as iron or copper. Excess generation of reactive oxygen species within tissues can damage DNA, lipids, proteins and carbohydrates. Which of these is the most important target of damage depends upon the cell type subjected to the oxidative stress and upon how it is imposed[33].

Free radicals of different forms are constantly generated for specific metabolic requirement and quenched by an efficient antioxidant network in the body. When the generation of these species exceeds the levels of antioxidant mechanism, it leads to oxidative damage of tissues and biomolecules, eventually leading to disease conditions, especially degenerative diseases.[34]

Many natural as well as synthetic coumarins and more complex related derivatives have recently drawn much attention due to its broad pharmacological activities include anti-bacterial, anti-thrombotic and vasodilatory, antimutagenic,lipoxygenase and cyclooxygenase inhibition, scavenging of reactive oxygen species, and anti-tumourigenic, appears to be based on the coumarin nucleus.[35,36]

Coumarin (2H-chromen-2-one) and its derivatives are widely distributed in nature and exhibit a broad pharmacological profile. A vast body of literature has accumulated recently linking the role of coumarin with several bioactivities, including: anticancer[37],anticoagulant, estrogenic, dermal, photosensitizing, antimicrobial, vasodilator, molluscacidal, antihelminthic, sedative, hypnotic, analgesic, hypothermic,[38,39] and free radical scavenging activity especially the superoxide anions generated by activated neutrophils.[40]

O.H.Hishmat and et.al. synthesized 3-acetyl coumarin and substituted derivatives.They also reported 3-(w-bromoacetyl) coumarin derivatives and 3-(w-bromoacetyl) coumarin with thiourea or with amines. They screened all compounds for antimicrobial activity.[41] K Srimanth and et.al., synthesized 3-((2-Hydrazino-4-thiazolyl) coumarino))phenyl methyl methines and 3-((2-Amino-4-thiazolyl) coumarino)) diaminomethines compounds by new method.[42] V Atitya Vardhan, and et.al reported condensation of 3-methyl/ethyl-5-mercapto-s- triazol with 3-acetyl coumarin and its derivatives.[43] N. Karal et.al. in their study, a new series of 4-(3-coumarinyl)-3-cyclohexyl-4-thiazoline-2-one benzylidene hydrazones were synthesized and were evaluated for antituberculosis activity against *Mycobacterium tuberculosis* H37Rv.[44]
Slobodan Sukdolak and et.al. in their study they reported, an easy and efficient procedure for the synthesis of eight 3-(thiazol-4-yl)-4-hydroxychromen-2-one derivatives. 3-Acetyl-4-hydroxychromen- 2-one was brominated with phenyltrimethylammonium tribromide to afford 3-(2-bromoacetyl)-4-hydroxychromen-2-

one which further allowed to reacts with thiourea, thioacetamide and ammonium dithiocarbamate to afford 3-(2-aminothiazol-4-yl)-4-hydroxychromen-2-one , 4-hydroxy-3-(2-methylthiazol-4-yl)chromen-2-one and 4-hydroxy-3-(2-mercaptothiazol-4-yl)chromen-2-one, respectively. In a similar manner, 3-(2-bromoacetyl)-4-hydroxychromen-2-one was treated with four mono-N-substituted thioureas and thiobenzamide to give the corresponding 3-(thiazol-4-yl)-4-hydroxychromen-2-one derivatives.[45]

K. R. Desai and et.al.,reported synthesis of 2-(3-hydroxy-4_ -methoxy phenyl)-3 -[4-(coumarin-3-yl)-thiazole-2-yl]-4 -thiazolidinone and 2-(3-hydroxy-4_ -methoxy phenyl)5-methyl-3 -[4-(coumarin-3-yl)-thiazole-2-yl]-4 -thiazolidinone through microwave method and screened for antimicrobial activity.[46]

Sarvesh Kumar and et.al., in their study different coumarin/thiocoumarin derivatives, like 7-hydroxy-4-methylcoumarin, 7,8-dihydroxy-4-methylcoumarin, 7-acetoxy-4-methylcoumarin, 7,8-diacetoxy-4-methylcoumarin, 7-hydroxy-4-methylthiocoumarin, 7,8-dihydroxy-4-methylthiocoumarin, 7-acetoxy-4-methylthiocoumarin and 7,8-diacetoxy-4-methylthiocoumarin were synthesized and evaluated for their effects on TNF-a induced expression of intercellular adhesion molecule-1 (ICAM-1) on endothelial cells and on NADPH-catalyzed rat liver microsomal lipid peroxidation with a view to identify modulators for expression of cell adhesion molecules and to establish structure–activity relationship. They found that dihydroxy and diacetoxy derivatives of thiocoumarin were more potent in comparison to the corresponding coumarin derivatives in inhibiting TNF-a-induced expression of ICAM-1.[47]

Irino o zhuravel; et.al ; developed solution phase synthesis of a combinatorial library of 3-(4-(coumarin-3-yl)-1,3-thiazol-2-carbomyl) propanoic acid amide.[48] K. R. Desai and et.al ; in their study they developed novel approach for rapid and efficient synthesis of heterocyclic Schiff bases and azetindinones under microwave irridation and screened for antibacterial activity.[49] K.Y.Anklekar and et.al ; developed electrophilic substitution reaction of bromoacetyl coumarin.[50] S. Sukdolak, et.al ; reported synthesis of 2-aminothiazole derivatives of 4-hydroxy-2H-chromen-2-one.[51]

K.R. Desai et.al ; reported synthesis of 2-N-Acetyl-4-(coumarin-3-yl)-thiazole ,2-N-(4-hydroxy-3-methoxyphenylchalconyl)- amino-4-(coumarin-3-yl)-thiazole , 5-(4-hydroxy-3-methoxyphenyl)-3-[4-(coumarin-3-yl)-thiazole-2-yl]-2-isoxazoline by conventional method and microwave method and screened for antimicrobial activity.[52]

K.R. Desai et.al ; reported synthesis of *N*-[4-(2-oxo-2*H*-chromen-3-yl)-1,3-thiazol-2-yl]acetamide, *N*-[4- (2- oxo-2*H*-chromen-3-yl)-1,3-thiazol-2-yl]cinnamamides,

2,3-dihydro-2-aryl-4-[4-(2-oxo-2*H*-chromen-3-yl)-1,3-thiazol-2-ylamino]-1,5-benzothiazipine through conventional method and microwave method.[53] P.Vijayakumar et.al reported synthesis procedure for 3-(2-(3-methyl-4,5-dihydro-furo(2,3-c) pyrazo-1-yl)-thiazol-4yl)-chromen-2-one.[54]

Nenad Vukovic and et.al in their studies series of imino and amino derivatives of 4-hydroxycoumarins were synthesized and evaluated for antioxidant potential, through different in vitro models such as (DPPH) free radical-scavenging activity, linoleic acid emulsion model system, reducing power assay and phosphomolybdenum method. Also, antimicrobial activity of obtained coumarins was evaluated against 13 bacteria and eightfungi.[55]

Afsheen Arshad and et.al synthesized two novel series of hydrazinyl thiazolyl coumarin derivatives were screened in vitro for antimicrobial activity against various bacteria species including Mycobacterium tuberculosis and Candida albicans.[56]

Wafaa S. Hamama and et.al in their study 3-acetylcoumarin was utilized as a key intermediate for the synthesis of 2-aminothiazole derivative via bromination of 3-acetylcoumarin to afford bromoacetyl coumarin followed by treatment with thiourea .Treatment of 2-aminothiazole derivative with 5-chloro-3-methyl-1-phenyl-1H-pyrazole-4-carbaldehyde,2-methyl-4H-benzo[d][1,3]oxazin-4-one, furo[3,4-b]pyrazine-5,7-dione or 2-methyl-5,6,7,8-tetrahydro-4Hbenzothieno[2,3-d][1,3]oxazin-4-one afforded diazine derivatives. Also, pyridopyrimidine was obtained via a one pot reaction of 6-aminothiouracil, p-chlorobenzaldehyde and 3-acetylcoumarin.Moreover, refluxing of 6-aminothiouracil with one equivalent amount of bromoacetyl coumarin afforded the thiazolopyrimidine, while the pyrrolothiazolopyrimidinez was revealed when two equivalent amounts of bromoacetyl coumarin was. Representative compounds of the synthesized products were evaluated as antioxidant agents.[57]

The coumarins are of great interest due to their biological properties. For their wide application, these compounds are attractive for further backbone derivatisation and screening as novel therapeutic agents.

AIM AND OBJECTIVES

In the pharmaceutical field, there is a need for new and novel chemical inhibitors of biological functions. Our efforts are focused on the introduction of chemical diversity in the molecular frame work in order to synthesizing heterocyclic molecules which can show antioxidant property.

The investigation was done in following manner:

1) Synthesis of 3-acetyl coumarin
2) Synthesis of bromoacetyl coumarin
3) Synthesis of chalcones of various aromatic aldehydes and ketones.
4) Substituted 3,5-Diphenyl-4,5-dihydropyrazole-1-carbothioamide preparation
5) Condensation of substituted 3,5-Diphenyl-4,5-dihydropyrazole-1-carbothioamide with bromo acetylcoumarin
4) Characterization of synthesized compounds using melting point, thin layer chromatography, UV, Infrared spectra, NMR spectra and mass spectra.
5) Evaluation of compounds for antioxidant activity.
6) Evaluation of compounds for antioxidant property in formulations.

Methods for synthesis:

3-Acetyl coumarin preparation: (3)

The compound 3- acetylcoumarin was prepared according to the procedure reported previously. A mixture of salicilaldehyde (0.01mol,) and ethyl acetoacetate (0.011 mol,) were mixed and few drops of piperidine were added. This mixture was stirred well for 2–3h at room temperature without any solvent. Reaction product was isolated by filtration. A yellow colored solid mass obtained. The product was recrystallised with ethanol and dried. The melting point of 3-acetylcoumarin is 120^0C.[41]

Bromoacetyl coumarin preparation: **(4)**

3-Acetylcoumarin (0.15 mol) was dissolved in acetic acid (25 mL) and a solution of bromine (0.15mol) in acetic acid (20mL) was added drop wise from a dropping funnel with constant stirring, after 4-5 h, a dark yellow solid separated. Purification by recrystallization (glacial acetic acid) gave 3-bromo acetylcoumarin off white needles, melting point 162 to165^0C

Substituted 3,5-Diphenyl-4,5-dihydropyrazole-1-carbothioamide preparation: (8)

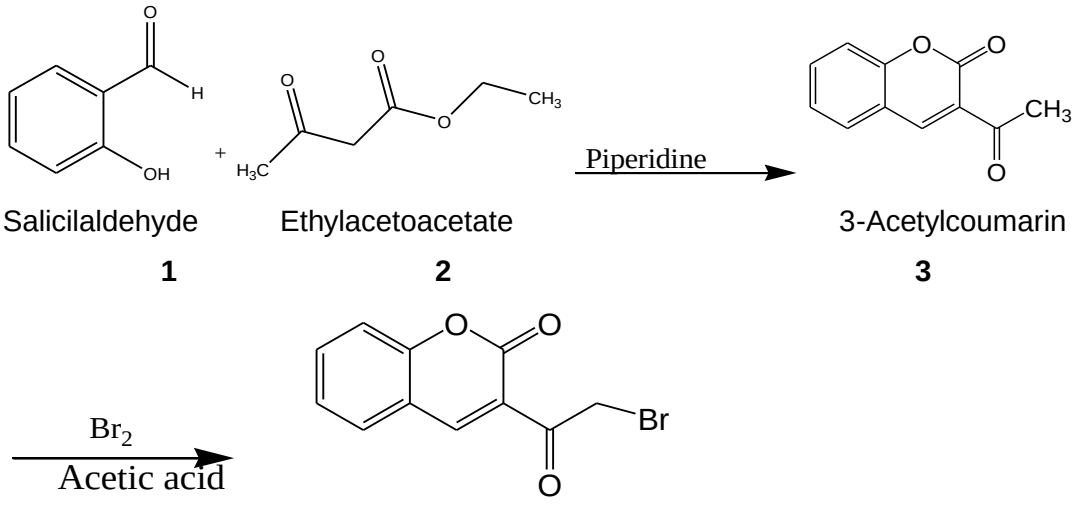
Substituted acetophenone (0.1mol) and sodium hydroxide (0.4g) were added in absolute alcohol and stirred at room temperature for 30 min. Substituted benzaldehyde (0.11 mol) was added drop wise in above solution and stirred well for 2-3-hrs at room temperature. Thiosemicarbazide (0.91g) was added and again reaction mixture was stirred for 12-15hrs at room temperature. After completion of reaction, reaction mixture was poured into ice cold water and neutralized with dil HCl. Reaction mixture was filtered and product was dried. Product was recrystallised with methanol.

Condensation of substituted 3,5-Diphenyl-4,5-dihydropyrazole-1-carbothioamide with bromo acetylcoumarin :(9)

Bromoacetyl coumarin (0.01 mol) was added in absolute alcohol and refluxed for 30 min. In boiling solution of bromoacetyl coumarin, substituted 3,5-Diphenyl-4,5-dihydropyrazole-1-carbothioamide(0.01 mol) was added and reaction mixture was refluxed for 3hrs. After completion of reaction product was filtered and recrystallised with methanol.

Scheme 1

Preparation of bromoacetyl coumarin

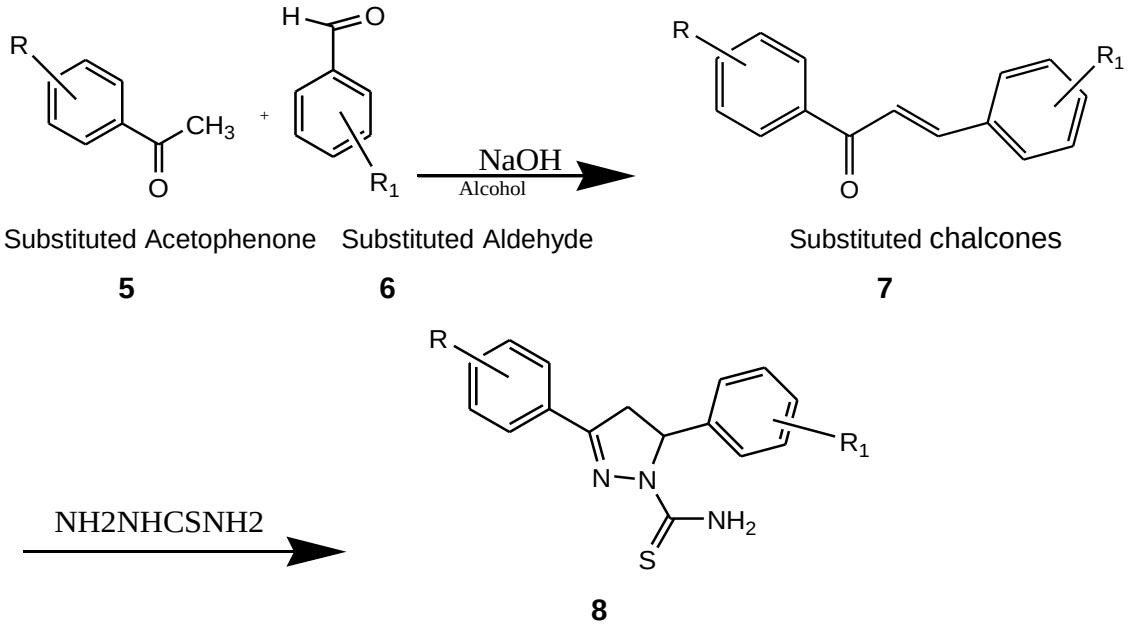


Salicilaldehyde **1** Ethylacetoacetate **2** 3-Acetylcoumarin **3**

Bromoacetyl coumarin **4**

Scheme 2

Preparation of Substituted 3,5-Diphenyl-4,5-dihydropyrazole-1-carbothioamide



Substituted Acetophenone **5** Substituted Aldehyde **6** Substituted chalcones **7**

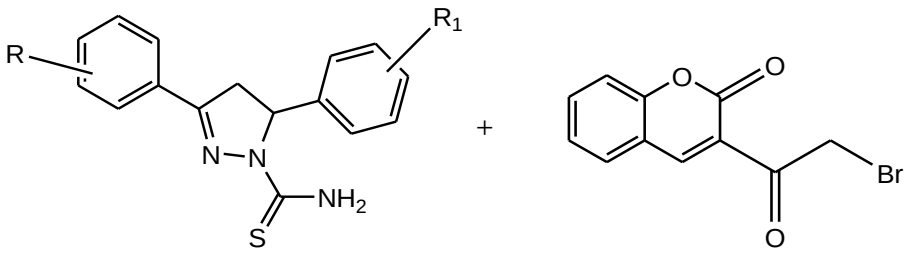
8

R=H, 2-OH,4-OH **R_1 = H, 4-OCH_3 ,2-OH, 4-OH**

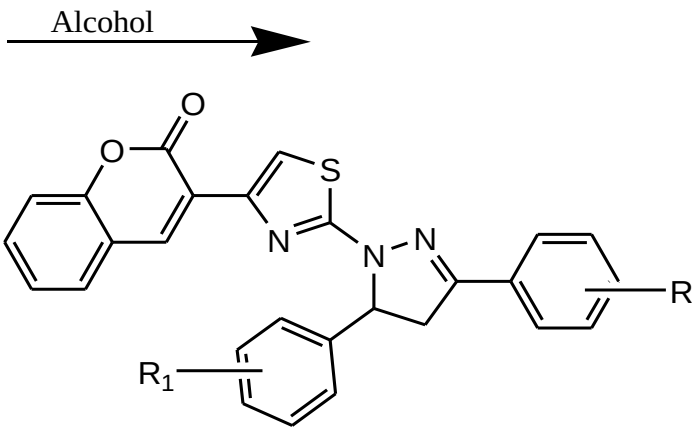
Scheme 3

Condensation of bromoacetyl coumarin (2) with substituted

3, 5-Diphenyl-4, 5-dihydropyrazole-1-carbothioamide (3)



8 + **4**



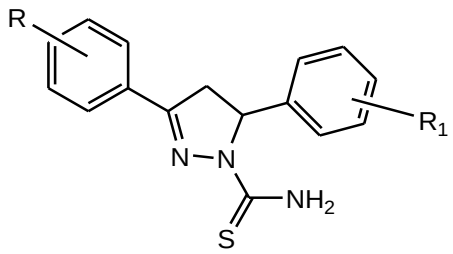
9

R=H, 2-OH,4-OH **R_1 = H, 4-OCH_3 ,2-OH, 4-OH**

Results and Discussion:

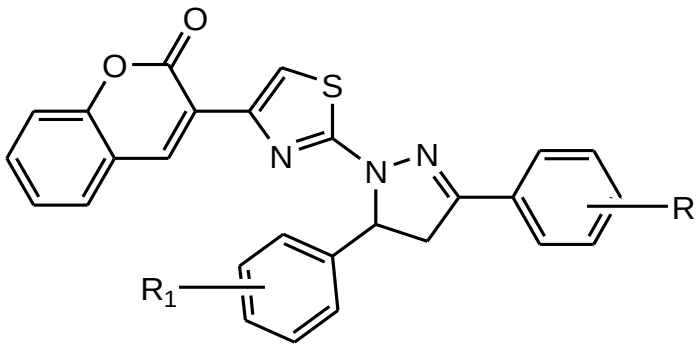
It is noteworthy that such a procedure for rapid preparation of various coumarin derivatives affords advantages of short reaction time, moderate yields and simple workup.

Table 1: Various *Substituted 3,5-Diphenyl-4,5-dihydropyrazole-1-carbothioamide*



Serial No	Substitution **R**	Substitution **R1**	Molecular formula	Molecular weight	Recrystallising solvent
1	H	H	$C_{16}H_{15}N_3S$	281.37	Methanol
2	H	2-OH	$C_{16}H_{15}N_3OS$	297.37	Methanol
3	H	4-OCH_3	$C_{17}H_{17}N_3OS$	311.40	Methanol
4	H	4-OH	$C_{16}H_{15}N_3OS$	297.37	Methanol
5	2-OH	H	$C_{16}H_{15}N_3OS$	297.37	Methanol
6	2-OH	2-OH	$C_{16}H_{15}N_3O_2S$	313.37	Methanol
7	2-OH	4-OCH_3	$C_{17}H_{17}N_3O_2S$	327.40	Methanol
8	2-OH	4-OH	$C_{16}H_{15}N_3O_2S$	313.37	Methanol
9	4-OH	H	$C_{16}H_{15}N_3OS$	297.37	Methanol
10	4-OH	2-OH	$C_{16}H_{15}N_3O_2S$	313.37	Methanol
11	4-OH	4-OCH_3	$C_{17}H_{17}N_3O_2S$	327.40	Methanol
12	4-OH	4-OH	$C_{16}H_{15}N_3O_2S$	313.37	Methanol

Table 2 : Various coumarin derivatives



Serial No	Substitution R	Substitution R1	Molecular formula	Molecular weight	Recrystallising solvent
1	H	H	$C_{27}H_{19}N_3O_2S$	449.52	Methanol
2	H	2-OH	$C_{27}H_{19}N_3O_3S$	465.52	Methanol
3	H	4-OCH_3	$C_{28}H_{21}N_3O_3S$	479.52	Methanol
4	H	4-OH	$C_{27}H_{19}N_3O_3S$	465.52	Methanol
5	2-OH	H	$C_{27}H_{19}N_3O_3S$	465.52	Methanol
6	2-OH	2-OH	$C_{27}H_{19}N_3O_4S$	481.52	Methanol
7	2-OH	4-OCH_3	$C_{28}H_{21}N_3O_4S$	495.52	Methanol
8	2-OH	4-OH	$C_{27}H_{19}N_3O_4S$	481.52	Methanol
9	4-OH	H	$C_{27}H_{19}N_3O_3S$	465.52	Methanol
10	4-OH	2-OH	$C_{27}H_{19}N_3O_4S$	481.52	Methanol
11	4-OH	4-OCH_3	$C_{28}H_{21}N_3O_4S$	495.52	Methanol
12	4-OH	4-OH	$C_{27}H_{19}N_3O_4S$	481.52	Methanol

Evaluation of antioxidant activity:

Antioxidant activity measured through reducing power by $FeCl_3$

- **Preparation of standard solution**

10 mg of ascorbic acid dissolved in 10 ml of distilled water. Make dilutions of this solution with distilled water to give 10, 30, 50, 60, 80 and 100 µg/ml solutions.

- **Preparation of test sample**

Required quantities of the test samples were dissolved in minimum quantity of methanol and volumes were made up to 10 ml with phosphate buffer. Separately all the samples were diluted in 10 ml volumetric flask with phosphate buffer to give 10, 30, 50, 60, 80 and 100 µg/ml solutions.

- **Preparations of reagents**

Phosphate buffer: 0.2 M phosphate buffer of pH 6.6 was prepared according to I.P. '2010

1 % Potassium ferricyanide solution: 2 gm of potassium ferricyanide was dissolved in 200 ml of distilled water

10 % Trichloro acetic acid: 40 gm of Trichloro acetic acid was dissolved in 400 ml of distilled water

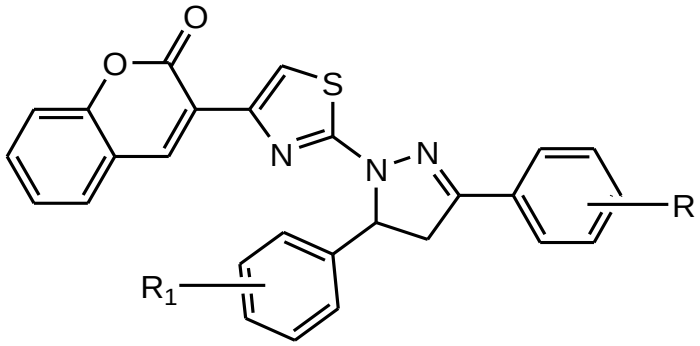
0.1 % Ferric chloride solution: 0.1 gm of ferric chloride was dissolved in 100 ml of distilled water.

- **Protocol for reducing power**

1. 2 ml of each sample and standard solutions were mixed separately with 2.5 ml of 1 % potassium ferricyanide solution.
2. This mixture was kept at 50^0C in water bath for 20 min.
3. After cooling 2.5 ml of 10 % Trichloro acetic acid, was added and centrifuged at 3000 rpm for 10 min.
4. 2.5 ml of supernatant was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1 % ferric chloride and kept for 10 min.
5. The absorbance of resulting solution was measured at 700 nm against control and those of samples were compared with the standard solution.

   Control was prepared in a similar manner excluding samples. [58]

Table 3: Following compounds were screened for antioxidant activity



Serial No	Substitution R	Substitution R1	Antioxidant activity
1	H	H	Tested for antioxidant activity
2	H	2-OH	Tested for antioxidant activity
3	H	4-OCH_3	Tested for antioxidant activity
4	H	4-OH	Tested for antioxidant activity
5	2-OH	H	Tested for antioxidant activity
6	2-OH	2-OH	Tested for antioxidant activity
7	2-OH	4-OCH_3	Tested for antioxidant activity
8	2-OH	4-OH	Tested for antioxidant activity
9	4-OH	H	Tested for antioxidant activity
10	4-OH	2-OH	Tested for antioxidant activity
11	4-OH	4-OCH_3	Tested for antioxidant activity
12	4-OH	4-OH	Tested for antioxidant activity

References:

1. Lyle MacWilliam,Modern Theories of Aging, MacWilliam Communications Inc.April 2002(www.macwillam.net)
2. Denham Harman, Aging: A Theory based on free radical and radiation chemistry,J Gerontol 11 (3): 1956,298-300.
3. Medvedev, Z.A., An attempt at a rational classification of theories of ageing. Biological Reviews of the Cambridge Philosophical Society 65,1990,375-398.
4. Abrams, W., Beers, M., Berkow, R., 1995. The Merc Manual of Geriatrics. Merck Research Laboratories, Whitehouse Station, NJ.
5. Beers, M.H., Berkow, R., Bogin, R.M., Fletcher, A.J., Rahman,M.I., 2000. The Merck Manual of Geriatrics,3rd ed. Merck Research Laboratories, Whitehouse Station, NJ.
6. Hayflick, L., 1987. Biological theories of aging. In: Maddox, 5. G. (Ed.), The Encyclopedia of Aging. Springer, New York.
7. Hayflick, L., Moorhead, P.,The serial cultivation of human diploid cell strains. Experimental Cell Research 25, 1961, 585-621.
8. Kirkwood, T.B.L., 1999. Time of Our Lives: The Science of Human Aging. Oxford University Press, Oxford *ISBN 978-0-19-512824-6*
9. Kirkwood, T.B.L., Understanding the odd science of aging. Cell 120, 2005,437-447.
10. Campisi, J., d'Adda, D.F., Cellular senescence: When bad things happen to good cells. Nature Reviews: Molecular Cell Biology 8, 2007, 729-740.
11. American Occupational Therapy Association, Occupational therapy practice framework: Domain and process, 2nd ed. American Journal of Occupational Therapy 62, 2008, 625-683.
12. Holliday, R., 2007. Aging: The Paradox of Life. Springer,Amsterdam.
13. Finkel, T., Serrano, M., Blasco, M.A., The common biology of cancer and ageing. Nature 448, 2007, 767-774.
14. Bird, A., Perceptions of epigenetics. Nature 447, 2007, 396-398.
15. Shringarpure, R., Davies, K.J.A., 2009. Free radicals and oxidative stress in aging. In: Bengtson, V.L., Gans, D., Putney, N.M., et al. (Eds.), Handbook of Theories of Aging, 2nd ed. Springer,New York..
16. Muller, F.L., Lustgarten, M.S., Jang, Y., Richardson, A.,Van Remmen, H., Trends in oxidative aging theories.Free Radical Biology & Medicine 43, 2007, 477-503.

17. Harman, D., Free radical theory of aging: An update. Increasing the functional life span. Annals of the New York Academy of Sciences 1067, 2006,10-21.
18. Ames, B.N. Delaying the mitochondrial decay of aging. Annals of the New York Academy of Sciences 1019, 2004, 406-411.
19. Cristofalo, V., 1988. An overview of the theories of biological aging. In: Birren, J., Bengtson, V. (Eds.), Emergent Theories of Aging. Springer, New York.
20. Finch, C.E., Ruvkun, G., The genetics of aging. Annual Review of Genomics & Human Genetics 2, 2001, 435-462.
21. Moody, H.R., 2010. Aging: Concepts and Controversies, 6th ed. Pine Forge Press, Thousand Oaks, CA.
22. Fraga, M.F., Ballestar, E.,Paz, M.F., Ropero, S.,Setien, F., Ballestar, M.L., et al.,. Epigenetic differences arise during the lifetime of monozygotic twins.Proceedings of the National Academy of Sciences of the United States of America 102,2005,10604-10609.
23. Fisher-Wellman K, Bloomer RJ. Acute exercise and oxidative stress: a 30 year history.*Dyn Med.* 2009 Jan 13 (online); 8: 1.
24. Brigelius-Flohé R. Commentary: oxidative stress reconsidered. *Genes Nutr.* 4(3): 2009, 161-163.
25. Beckman KB, Ames BN. The free radical theory of aging matures. *Physiol Rev* 78: 1998,547-581.
26. Gilca M, Stoian I, Atanasiu V, Virgolici B. The oxidative hypothesis of senescence. *JPostgrad Med*. 53: 2007, 207-13.
27. Fukui H, Moraes CT. The mitochondrial impairment, oxidative stress and neurodegeneration connection: reality or just an attractive hypothesis? *Trends Neurosci.* 31(5); 2008, 251-256.
28. Halliwell BH, Gutteridge JMC. *Free Radicals in Biology and Medicine.* Oxford, UK:Oxford Univ. Press, 1989
29. Fahn S, Cohen G. The oxidant stress hypothesis in Parkinson's disease: evidence supporting it. *Ann Neurol* 32: 1992, 804-812.
30. Pezzuto, J. M., Jung, E., 2007. Autooxidation and antioxidants. In Swarbrick,J.(Eds.), Encyclopaedia of Pharmaceutical Technology 3rd edition, volume 1, Park informa health care, New York, 139-154.
31. Percivil, M.,1998. Antioxidants. Clinical Nutrition Insights,1-4.

32. Nema, S., Brandel, R. J., Waskahn, R., 2007. Excipients: Parenteral dosage forms and their role. In Swarbrick,J.(Eds.), Encyclopaedia of Pharmaceutical Technology 3rd edition, volume 3, Park informa health care, New York ,1625-1627.
33. Aruoma OI, Kaur H, Halliwell B. Oxygen free radicals and human diseases.J.R.SOC.Health, 111(5): 1991, 172-7.
34. Gutteridge JMC. Free radicals in Disease Processes: A Compilation of cause and consequence. Free radic. Res. Comm, 19: 1995,141.
35. Mohammad I., Zafar I., Javid H, Hidayat H, Manzoor A, Asma E, Muhammad I.C.Chemical constituents and antioxidant activity of Geranium Wallichianum. Rec.Nat. Prod. 3(4): 2009, 193-197.
36. Bondet V., Brand-Williams W., Berset C. Kinetics and mechanisms of antioxidant activity using the DPPH free radical method. Food Science and Technology. 30: 1997,609-615.
37. Murray RDH, Mendez J, Brown SA. The natural coumarins: occurrence, chemistry and biochemistry. New York: John Wiley; 1982. 282-289.
38. Soine TO. Naturally occurring coumarins and related physiological activities J Pharm Sci 53, 1964,231.
39. O'Kennedy R, Thornes RD, editors. Coumarins biology, applications and mode of action. Chichester: John Wiley; 1997. 125-141.
40. Paya M, Goodwin PA, De Las Heras B, Hoult RS. Superoxide scavenging activity in leukocytes and absence of cellular toxicity of a series of coumarins. Biochem Pharmacol 48: 1994,445-451.
41. Hishmat O.H., J.A.A. Miky, A.A. Farrag, and E.M. Fadl-Allah Synthesis of Some Coumarin Derivatives and their Antimicrobial Activity *Arch. Pharm. Res.* 12(3), 1989,181-185
42. Srimanth K. and V Rajeswar Rao; Synthesis of some new type of thiazolyl coumarin; Indian journal of chemistry Vol 38, 1999, 473-475
43. Atitya Vardhan V., V Ravi Kumar and V Rajeswar Rao ; Condensation of 3-methyl/ethyl-5-mercapto-s- triazol with 3-acetyl coumarin and its derivatives.; Indian journal of chemistry Vol 38B, 1999, 18-23
44. Karalı N., A. Kocabalkanlı, A. Gursoy, O. Ates; Synthesis and antitubercular activity of 4-(3-coumarinyl)-3-cyclohexyl-4-thiazolin-2-one benzylidenehydrazones ; Il Farmaco 57, 2002, 589–593

45. Slobodan Sukdolak, Slavica Soluji,Nenad Vukovi, Nedeljko Manojlovi and Ljubomit krsti , Synthesis of some 3-(thiazol-4-yl)-4-hydroxycoumarins *J.Serb.Chem.Soc. 69*(5), 2004,319–326
46. Naik B.D., and K. R. Desai; Microwave Assisted Heterocyclization: A Rapid and Efficient Synthesis and Antibacterial Activity of Novel Thiazolidinones E-Journal of Chemistry Vol. 1, No. 5,2004, 263-266
47. Sarvesh Kumar, Brajendra K. Singh, Neerja Kalra, Vineet Kumar, Ajit Kumar,Ashok K. Prasad,Hanumantharao G. Raj, Virinder S. Parmarb and Balaram Ghosha Novel thiocoumarins as inhibitors of TNF-α induced ICAM-1 expression on human umbilical vein endothelial cells (HUVECs) and microsomal lipid peroxidationq Bioorganic & Medicinal Chemistry 13,2005,1605–1613
48. Irino o zhuravel; Sergiy M Kovalenko; Sergiy V. Vlsavo and Valentine P., Chernykh ,Solution phase synthesis of a combinatorial library of 3-(4-(coumarin-3-yl)-1,3-thiazol-2-carbomyl) propanoic acid amide; Molecules 2005, 10, 444-456
49. Naik B., and K. R. Desai ; Novel approach for rapid and efficient synthesis of heterocyclic Schiff bases and azetindinones under microwave irridation ; Indian journal of chemistry Vol 45 B, 2006, 267-271
50. Anklekar K.Y., R.A Kausnaur V.M. Kulkarni ;Electrophilic substitution reaction of bromoacetyl coumarin;Indian journal of chemistry Vol 45B ,2006, 325-327
51. Sukdolak S., N. Vukovi, S. Soluji, M. Miloev, N. Manojlovi, LJ. krsti ; Synthesis of new 3-(2-aminothiazol-4-yl)-4-hydroxy-2*H*-chromen-2-one derivatives; *J. Serb. Chem. Soc. 71* (6) 2006,581–585
52. Desai J.T., C.K. Desai and K.R. Desai A Convenient, Rapid and Eco-Friendly Synthesis of Isoxazoline Heterocyclic Moiety Containing Bridge at 2°-Amine as Potential Pharmacological Agent*J. Iran. Chem. Soc.,* Vol. 5, No. 1, 2008, 67-73
53. Jignesh P. Raval, Jignasu T. Desai, Chintan K. Desai, and Kishor R. Desai A comparative study of microwave assisted and conventional synthesis of 2,3-dihydro-2-aryl-4-[4-(2–oxo–2H–chromen–3–yl)–1,3-thiazol–2–ylamino]-1,5–benzothiazepines and its antimicrobial activity ARKIVOC 2008 (xii) 233-244

54. P Vijayakumar, K.Manoher Reddy, V.Rajeswar Rao; Zinc chloride catalyzed one pot synthesis of 3(-2-(3 –methyl-4,5-dihydro furo(2,3-c)-pyrazol-1-yl)-thiazoyl-4-yl) chromen-2-ones via three component reaction, ; Indian journal of chemistry Vol 49 B, 2010, 836-839
55. Nenad Vukovic , Slobodan Sukdolak, Slavica Solujic, Neda Niciforovic Substituted imino and amino derivatives of 4-hydroxycoumarins as novel antioxidant, antibacterial and antifungal agents: Synthesis and in vitro assessments, Food Chemistry 120 ,2010, 1011–1018
56. Afsheen Arshad , Hasnah Osman , Mark C. Bagley, Chan Kit Lam, Suriyati Mohamad, Anis Safirah Mohd Zahariluddin , Synthesis and antimicrobial properties of some new thiazolyl coumarin Derivatives, European Journal of Medicinal Chemistry 46 ,2011, 3788-3794
57. Wafaa S. Hamama, Moged A. Berghot, Eman A. Baz, and Moustafa A. Gouda Synthesis and Antioxidant Evaluation of Some New 3-Substituted Coumarins ,Arch. Pharm. Chem. Life Sci., 344, 2011, 710–718
58. Oyaizu M. Studies on product of browning reaction: antioxidative activities of products of browning reaction prepared from glucosamine. *Jpn. J. Nutr*. 44, 1986: 307-315.

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